Apolipoprotein B-48 and retinyl palmitate are not equivalent markers of postprandial intestinal lipoproteins

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Abstract This study compared retinyl palmitate and apolipoprotein (apo) B-48 as markers of postprandial triglyceride-rich lipoproteins. Nine non-diabetic men received an oral vitamin A-containing fat load. We measured retinyl palmitate, apoB-48, apoB-100, and triglyceride levels in $S_f > 400$, S_f 60–400 and S_f 20–60 lipoproteins. The peak retinyl palmitate concentration was delayed compared to the peak apoB-48 concentration in each fraction. The discrepancy between retinyl palmitate and apoB-48 was further investigated in another study of 12 men. In that study, a fat load was given and 5 h later, lipolysis was stimulated in vivo with heparin (60 U/kg, i.v.) and the same parameters were followed. Thirty minutes after heparin, triglyceride levels decreased significantly in the three triglyceride-rich lipoprotein fractions ($S_f > 400$, S_f 60–400 and S_f 20–60). ApoB-48 levels also fell significantly in the three triglyceride-rich lipoprotein fractions. In contrast, retinyl palmitate concentrations did not change significantly in $S_f > 400$ and S_f 60–400 fractions and increased significantly in the S_f 20–60 fraction. Our results indicate that retinyl palmitate and apolipoprotein B-48 do not mark the same properties of postprandial intestinal lipoproteins. The metabolic pattern of apolipoprotein B-48 parallels that of triglyceride. One possible explanation for these observations is that the apoB-48-containing triglyceride-rich lipoproteins are metabolically heterogeneous and that older particles, those in circulation for a longer period of time, may be cleared more rapidly than newer ones.—Lemieux, S., R. Fontani, K. D. Uffelman, G. F. Lewis, and G. Steiner. **Apolipoprotein B-48 and retinyl palmitate are not equivalent markers of postprandial intestinal lipoproteins. *J. Lipid Res.* 1998.** 39: **1964–1971.**

Supplementary key words postprandial lipoproteins • heparin • lipolysis stimulation

There is increasing support for the concept that a high fasting concentration of plasma triglyceride (TG) is related to an increased risk of coronary artery disease (CAD) (1). Because humans normally spend more time in the postprandial state than in the fasting state, there has been much interest in the metabolism of lipoproteins after the ingestion of a fat-rich meal. In that regard, it has been demonstrated that increased plasma levels of postprandial lipids are associated with an increased risk of CAD (2–6).

The metabolism of postprandial lipoproteins is commonly studied after the ingestion of a fat load supplemented with vitamin A. The lipoprotein retinyl esters, derived from vitamin A, have been used as a marker of intestinal lipoproteins. There are three reasons for this: vitamin A is incorporated into lipoproteins as retinyl palmitate (RP) by the intestine during the absorption of fat; RP exchange between lipoproteins is thought to be minimal, particularly during the first 8 to 12 h after a fat load (7–9); and after the hepatic uptake of RP-containing lipoprotein, RP is not resecreted as a lipoprotein component (10, 11). Therefore, RP is thought to remain associated with the same lipoprotein from its secretion by the intestine until its uptake by the liver.

Apolipoprotein (apo) B-48 is the lower molecular weight form of apoB which, in humans, is produced only by the intestine (12). One molecule of apoB-48 is present per TG-rich lipoprotein (13). It remains associated with intestinal lipoproteins throughout the lipolytic cascade and is not exchanged with other lipoproteins (12, 14). Therefore, it can serve as a marker of the particles of TG-rich lipoproteins of intestinal origin in humans (12). Theoretically, both apoB-48 and RP should remain associated with intestinal lipoproteins, from their secretion until their uptake by the liver. The former should mark the particle of intestinal TG-rich lipoprotein and the latter should mark its lipid. However, some studies have shown that after the ingestion of a high-fat meal, the peak RP concentration was delayed compared to the peak of apoB-48 (15–17). The mechanisms that might explain the differences observed in the pattern of changes in RP and

Abbreviations: TG, triglyceride; RP, retinyl palmitate; apo, apolipoprotein; HPLC, high performance liquid chromatography; CAD, coronary artery disease; LPL, lipoprotein lipase.

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apo-B48 concentrations in the postprandial state remain to be clarified.

We report two studies. The first (postprandial study) examined the TG-rich lipoproteins over an 8-h period after a standard high fat load. Because of its results, a second study (postheparin study) was undertaken. It examined the clearance of postprandial lipoproteins by giving a high fat load and, at the approximate peak time of TG-rich lipoproteins levels, stimulating their clearance by increasing lipoprotein lipase (LPL) activity with heparin. In both studies, we measured and compared the changes in RP, apoB-48, apoB-100, and TG in subfractions of TG-rich lipoproteins.

METHODS

Subjects

For the postprandial study, 9 healthy men were recruited from the general population by advertising in the University of Toronto newspaper. None had diabetes. All had normal plasma TG levels. None were taking any medication known to affect lipid metabolism. Twelve men without diabetes were recruited from the outpatient Endocrinology Clinic at the Toronto Hospital for the second study (postheparin study). The group's mean TG level was 3.4 ± 0.3 mmol/L and ranged from 2.1 to 5.3 mmol/L.

Weight and height were recorded and body mass index as well as body surface area were calculated. Waist and hip circumferences were measured and the waist-to-hip ratio was calculated. Characteristics of the two groups of subjects are presented in **Table 1**. Each participant signed an informed consent in accordance with guidelines of Human Ethics Committee of the Toronto Hospital.

Protocol: postprandial study

For the postprandial study, subjects were admitted to the Clinical Investigation Unit of the Toronto Hospital in the morning after an overnight fast and abstention from alcohol for 48 h. After obtaining a fasting blood sample, the subjects ingested, within 10–15 min, a high fat load that resembled a milkshake. It contained soybean oil (50 g/m^2 of body surface), dried egg-white (25 g/m^2), dried egg-yolk (6.3 g/m^2) anhydrous dextrose (50 g/m^2), distilled water (200 mL), and banana extract (10 g). Water-soluble vitamin A (60,000 IU/m^2: Aquasol A, Astra Pharmaceuticals, Westborough, MA) was added to the fat load. Subjects were allowed to walk during the test and to drink water. No food or smoking was permitted until the end of the study. Blood samples were obtained through an intravenous catheter that had been inserted into a forearm vein. Samples were taken just before (baseline) and at 2, 4, 6 and 8 h after the ingestion of the high fat load.

TABLE 1. Characteristics of subjects participating in the studies

Variables	Postprandial Study (n = 9)	Postheparin Study (n = 12)
Age (years)	56.1 ± 2.0	47.3 ± 3.5
Body mass index (kg/m^2)	24.8 ± 0.6	30.3 ± 2.1
Waist-to-hip ratio[a]	0.92 ± 0.01	0.95 ± 0.01
Fasting plasma TG (mmol/L)	1.6 ± 0.2	3.4 ± 0.3

Values are means ± SEM. TG, triglyceride.
[a] Measured in all subjects in the postprandial study and in 9 subjects in the postheparin study.

Protocol: postheparin study

For the postheparin study, a group of 12 men (see subject's characteristics in Table 1) were examined.They were also admitted at the Clinical Investigation Unit of The Toronto Hospital in the morning after an overnight fast and abstention from alcohol for 48 h. They ate a high fat load containing 60 g of fat/m^2 of body surface. Of the energy, 67.9% was from fat (polyunsaturated/saturated ratio of 0.526), 23.7% from carbohydrate, and 10.4% from protein. The fat load consisted of a high fat milk shake containing ice cream, cream, peanut butter, and corn oil and a toasted cheese sandwich. The fat load was supplemented with water-soluble vitamin A (60,000 IU/m^2). Five hours after the ingestion of the meal, they received by intravenous injection 60 U heparin sodium/kg of body weight (Organon Tieknika, Toronto, Canada) to stimulate the hydrolysis of TG in vivo. Blood samples were taken just before and then 15 min and 30 min after the injection of heparin.

Blood sampling

Blood samples were drawn into Vacutainer tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ) containing Na_2EDTA (4 mmol/L) which were immediately placed on ice. Tubes were covered with aluminium foil in order to protect the samples from the light. Plasma was then recovered within 30 min by centrifugation (1500 rpm, for 20 min at 4°C). Phenylmethylsulfonylfluoride and aprotinin (0.45 μL and 2.25 μL per mL of plasma, respectively) were immediately added.

Plasma ultracentrifugation

Ultracentrifugation of each plasma sample was performed in order to obtain three fractions of TG-rich lipoproteins ($S_f > 400$, S_f 60–400, S_f 20–60). We used a modification of a procedure previously reported (18). Plasma was overlayered with a solution of d 1.006 g/mL. All samples were first spun at 39,000 rpm in a 70.1 Ti Rotor (Beckman Instruments, Inc, Palo Alto, CA), for 16 h at 15°C to obtain the TG-rich lipoprotein fraction ($S_f > 20$). Consecutive runs were then performed in a SW-40 bucket rotor (Beckman Instruments, Inc, Palo Alto, CA) to obtain the $S_f >$ 400 fraction (12,500 rpm for 30 min at 15°C) and then the S_f 60–400 (top) and S_f 20–60 fractions (bottom) (29,000 rpm for 2 h at 15°C).

Determination of apoB-100 and apoB-48 concentrations

The total amount of apoB in the isolated subfractions ($S_f >$ 400, S_f 60–400 and S_f 20–60) was quantified with an electroimmunoassay that had been developed to permit accurate measurements of apoB in TG-rich lipoproteins (19). In our studies, the recovery of plasma apoB in the fractions isolated was 96.8 ± 15.8%. The relative amount of apoB-48 and apoB-100 in the sample was determined by SDS-PAGE using rod gels as previously described (18). Samples containing 200–500 μg total lipoprotein were first delipidated using the methanol–chloroform–diethyl ether method (20). The delipidated precipitate was dissolved overnight in sample buffer (sodium phosphate at 50 mm, SDS at 1%, and mercaptoethanol at 1%, pH 7.2). Four dilutions of the delipidated solutions were then applied to 3.3% SDS-PAGE rod gels. Gels were stained with Coomassie Brilliant Blue R-250 (Eastman-Kodak Co., Rochester, NY) for 24 h, and destained for 3 days in 10% acetic acid. Gels were scanned with a densitometer (ImageMaster DTS, Pharmacia-LKB) connected to a personal computer equipped with software that allowed integration of areas under the scanning curves. Limits for beginning and end of each integration were set for each band. For each gel, a line was drawn that separated the background from the actual area under each peak and the background was then subtracted automatically. A regression equation relating the dilution factor of the

sample to the intensity of staining was calculated for apoB-48 and apoB-100 separately, for each sample. The relative mass of apoB-48 to apoB-100 was obtained by multiplying the ratio of the slopes (apoB-48/apoB-100) by a factor that took into account the relative chromogenecities of the two apolipoproteins (0.57) (18). The absolute masses of apoB-48 and apoB-100 were calculated from their relative quantities and the total apoB mass was measured by electroimmunoassay.

Retinyl palmitate measurements

To avoid denaturing the light-sensitive RP, samples were protected from the light during all the procedures leading up to the measurement of RP. Samples of 400 μL were used for RP determination. Retinyl acetate (Sigma, R-4632, St. Louis, MO) was used as an internal standard and was added in a known concentration to each sample. A lipid extraction (21) was performed with methanol (2 mL) and chloroform (2 mL). After mixing, an additional 2 mL of chloroform was added. The solution was centrifuged for 5 min at 1500 rpm at 4°C. The upper phase was removed and the lower phase was filtered and dried under nitrogen. Lipids were then dissolved in 200 μL of chloroform–methanol 2:1, of which 40 μL was injected into a high-performance liquid chromatography (HPLC) system (Hewlett-Packard, model 1050) equipped with a Supelcosil reverse phase column (25 cm × 0.46 mm ID, Supelco CO., Mississauga, Ontario). A solvent mixture of isopropanol–acetone 1000:1.25 was used as the mobile phase while a mixture of methanol–ammonium acetate (60 mm)–acetone 850:150:1.25 constituted the buffer within the mobile phase. The mobile phase and the buffer were pumped into the column at a constant rate of 1.25 mL/min. The proportion of each mixture was 50% for the first 5 min and then was gradually changed to 70% (mobile phase) and 30% (buffer) until the end of the HPLC run which lasted 20 min. After elution from the column, each sample was read by a spectrophotometer at 330 nm. The area under the peaks of RP and of the internal standard was calculated. RP concentration was obtained by taking into account the concentration of the standard, the area under the peaks of RP and of the standard as well as their respective molecular weight. The recovery of plasma RP in the lipoprotein fractions was 81.4 ± 12.3%.

Triglyceride measurements

TG concentrations were measured in each subfraction by using a commercial kit (Triglycerides/GB; Boehringer Mannheim Biochemicals, Laval, Québec).

Assessment of RP in the TG-rich apoB-100-containing lipoproteins

In four subjects participating in the postheparin study, blood was drawn just before the injection of heparin (5 h after the ingestion of the high fat load). The plasma was immediately separated and the TG-rich lipoproteins ($S_f > 20$) were isolated by ultracentrifuging this plasma at d 1.006 g/mL. It was then applied to an immunoaffinity column to separate the apoB-100-containing lipoproteins. The immunoadsorbent was the monoclonal antibody 5E11 (provided by Dr. Y. Marcel, Ottawa Heart Institute, Ottawa, Canada), which is specific for an epitope located between apoB-100 residues 3441–3569. Sepharose 4B was activated and 200 mg of the antibody (5 mg/mL) was coupled to 50 mL of CNBr activated Sepharose 4B according to the procedures used by Milne, Weech, and Marcel (22). A volume of 3.0 mL of TG-rich lipoprotein was applied to an 8 mL column of 5E11 Sepharose. Bound lipoproteins (apoB-100-containing lipoproteins) were eluted with 3 m sodium thiocyanate. The eluted fraction was then desalted and concentrated (Centriplus, Amicon, Beverly, MA). The fraction recovered was run on SDS gel to confirm the absence of apoB-48 in the eluate. The RP content of this TG-rich apoB-100-containing lipoprotein fraction was then measured as described above. As with all procedures involving RP, these were conducted in the absence of light.

Statistical analyses

In the first study, variables were measured at 5 time points (0, 2, 4, 6, and 8 h after the high fat load) while in the second study, measurements were performed at 3 time points (before heparin injection, 15 min and 30 min postheparin). For both studies, one-way ANOVA on repeated measurements was performed to detect the effect of time on variables measured. In the presence of a significant time effect, multiple comparisons (Duncan multiple range test) were used to identify specifically which time points show significant differences for variables measured.

Times at which peak concentrations were achieved during the postprandial period for RP, apoB-48, and TG were compared using an analysis of variance followed by multiple comparisons (Duncan multiple range test). In addition, differences in concentrations of lipoprotein components (RP, apoB-48, apoB-100, and TG) between each subfractions ($S_f > 400$, S_f 60–400, S_f 20–60) were tested at each time point, also using an analysis of variance followed by multiple comparisons. The critical *P* value for significance was set at 0.05. All the analyses were performed with the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

Postprandial study

Figure 1 shows concentrations of RP, apoB-48, apoB-100, and TG in each TG-rich lipoprotein fraction at baseline and 2, 4, 6, and 8 h after the ingestion of the high fat meal. The asterisks indicate those values that are significantly different from the t_0 values. After 8 h, values of TG and RP were still different from baseline in all three fractions. ApoB-48 concentrations measured at 8 h in $S_f > 400$ and S_f 20–60 had returned to values that were not different from baseline levels, whereas the concentration measured at 8 h in S_f 60–400 was still significantly higher than baseline concentrations. ApoB-100 levels measured in $S_f > 400$ and S_f 60–400 at 8 h were significantly higher than baseline concentrations, whereas apoB-100 levels measured in the S_f 20–60 did not change with time after the fat load (Fig. 1).

Both fasting and after the fat load, TG concentrations were the highest in the S_f 20–60, intermediate in the S_f 60–400 and the lowest in $S_f > 400$. No significant differences were found among the TG concentrations in the three TG-rich lipoprotein fractions at the time of their peak levels, i.e., 4 and 6 h after the fat load. ApoB-48 and apoB-100 levels were the highest in the S_f 20–60, intermediate in the S_f 60–400, and the lowest in $S_f > 400$, in the fasting state and in response to the fat load. On the other hand, after the fat load, RP levels were the lowest in the S_f 20–60 fraction, intermediate in $S_f > 400$, and the highest in the S_f 60–400 fraction.

For each subject, and in each fraction, the time point (0, 2, 4, 6, or 8 h) at which the concentration of RP, apoB-48, or TG was the highest was determined (peak value). **Table 2** shows that the peak in RP concentrations in the

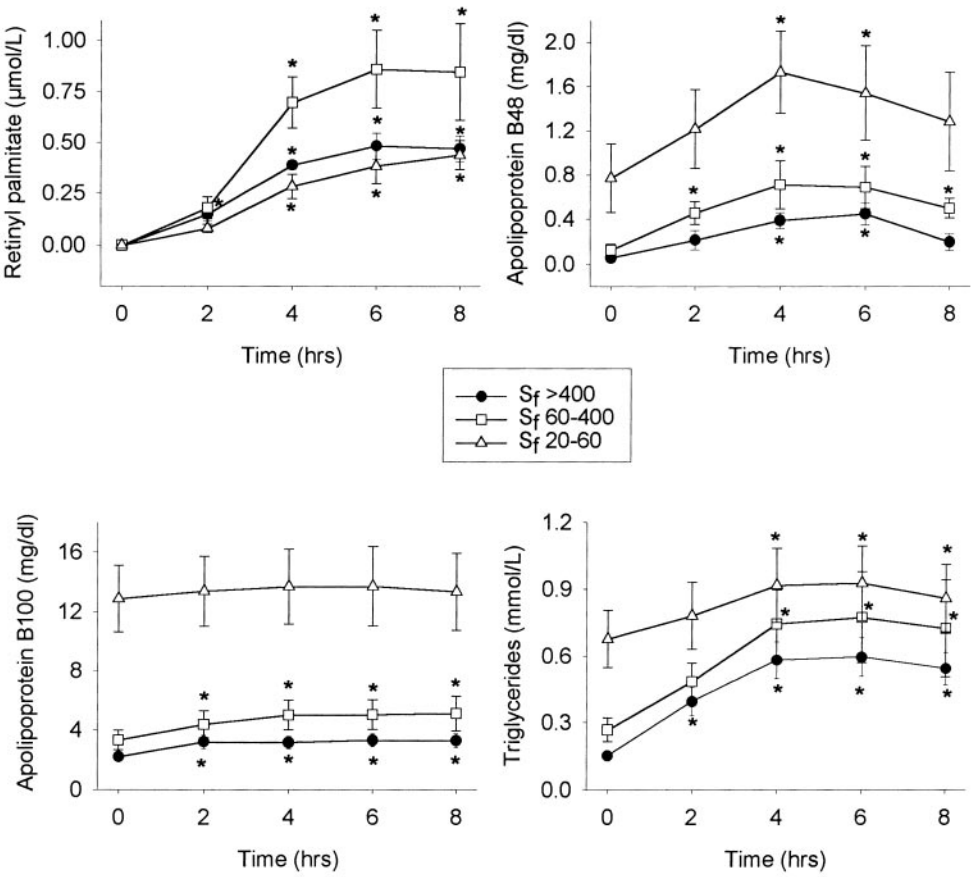

Fig. 1. Changes in retinyl palmitate, apolipoprotein B-48, apolipoprotein B-100, and triglyceride concentrations after the ingestion of the high fat load. Concentrations were measured in the three fractions of triglyceride-rich lipoproteins ($S_f < 400$, S_f 60–400, and S_f 20–60). Data are means ± SEM. *Significantly different from baseline value, $P < 0.05$.

three fractions occurred later than the peak of the other lipoprotein components. However, the difference was statistically significant only in the S_f 20–60 fraction. In every fraction, peaks in apoB-48 and TG levels were reached essentially at the same time.

Postheparin study

To examine further the differences in the behavior of the components of the TG-rich lipoproteins, we undertook another study, the postheparin study. Participants received a fat load in order to generate postprandial TG-rich lipoproteins. Five hours later, they received heparin to promote the clearance of these lipoproteins. The concentrations of RP, apoB-48, apoB-100, and TG in $S_f > 400$, S_f 60–400, and S_f 20–60 were measured just before the injection of heparin and then 15 min and 30 min later. TG and RP levels were also measured in the plasma before and after heparin. Plasma TG levels fell from 6.51 ± 0.51 mmol/L (before heparin) to 3.51 ± 0.42 mmol/L (30 min after heparin) ($P < 0.05$). On the other hand, there was a redistribution of RP among the TG-rich lipoprotein fractions but no change in plasma RP was observed after heparin (11.1 ± 0.9 μmol/L, before heparin, 11.7 ± 1.2 μmol/L, 30 min after heparin). Absolute values for changes in TG, apoB-48, and RP are presented in **Fig. 2**.

Before heparin, TG concentrations were the highest in the S_f 60–400 fraction, intermediate in the S_f 20–60 and the lowest in $S_f > 400$. However, these differences were not significant. ApoB-48 and apoB-100 levels were the highest in the S_f 20–60, intermediate in the S_f 60–400, and the lowest in $S_f > 400$ whereas RP concentrations were the highest in the S_f 60–400 fraction, intermediate in $S_f > 400$, and the lowest in the S_f 20–60 fraction, before heparin injection. TG levels decreased significantly in all three fractions 15 min after heparin injection and did not change significantly thereafter (Fig. 2). ApoB-48 concentrations also decreased significantly after heparin in all

TABLE 2. Comparisons between retinyl palmitate, apolipoprotein B-48, apolipoprotein B-100, and triglyceride peaks during the 8-h fat load test in the sample of 9 men

	Peak (hours)		
	RP	ApoB-48	TG
$S_f > 400$	6.2 ± 0.5	5.1 ± 0.5	5.1 ± 0.6
S_f 60–400	6.7 ± 0.5	5.6 ± 0.6	5.6 ± 0.6
S_f 20–60	7.1 ± 0.5	4.9 ± 0.4[a]	4.4 ± 0.9[a]

Values are means ± SEM. RP, retinyl palmitate; apo, apolipoprotein; TG, triglyceride.
[a]Significantly different from RP peak ($P < 0.05$).

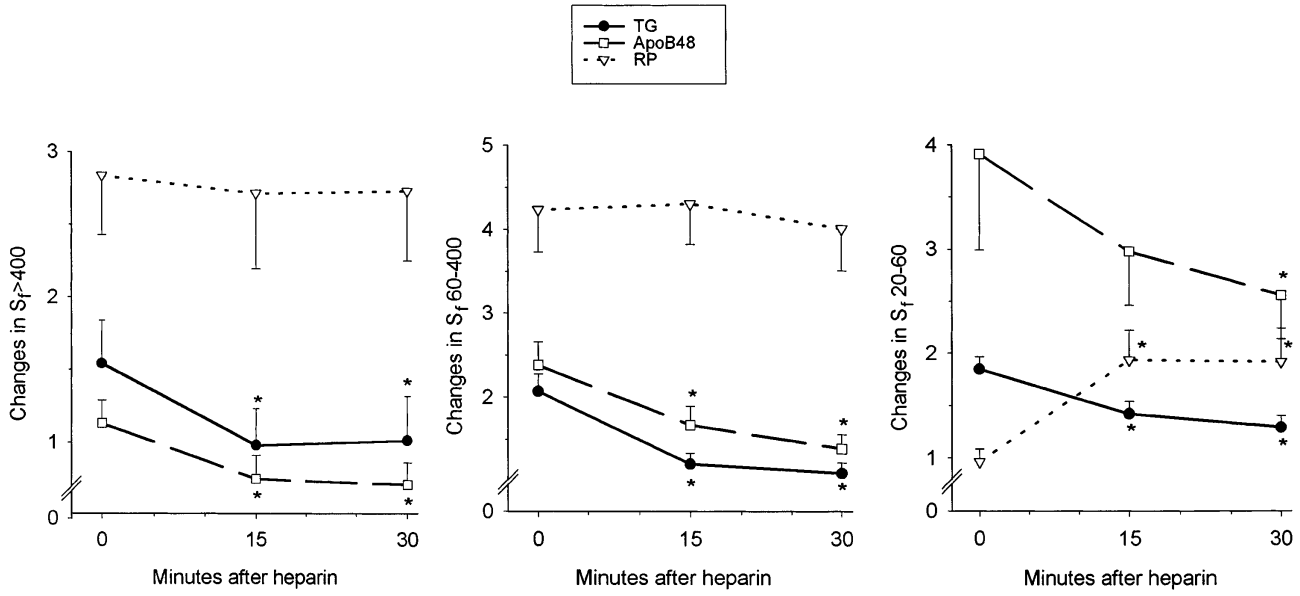

Fig. 2. Changes in retinyl palmitate, apolipoprotein B-48, and triglyceride concentrations after the injection of heparin (5 h after the ingestion of a high fat meal). Concentrations were measured in three fractions of triglyceride-rich lipoproteins ($S_f < 400$, S_f 60–400, and S_f 20–60). Units are mmol/L for TG, mg/dl for apoB-48, and μmol/L for RP. *Significantly different from the value measured at baseline, $P < 0.05$.

TG-rich lipoprotein fractions. These apoB-48 changes paralleled those observed for TG levels. RP levels after heparin were in marked contrast to the apoB-48 and TG levels noted above. The RP did not change significantly in the $S_f > 400$ and S_f 60–400 fractions and actually increased in the S_f 20–60 fraction (Fig. 2). ApoB-100 concentrations measured at 15 and 30 min postheparin were not significantly different from pre-heparin values in $S_f > 400$ (6.2 ± 0.8 mg/dL) and S_f 20–60 (29.1 ± 2.2 mg/dL). There was a slight decrease of the apoB-100 levels in the S_f 60–400 fraction after heparin (11.3 ± 1.1 mg/dL at t = 0; 10.1 ± 1.1 mg/dL at 30 min postheparin, $P < 0.05$; not shown).

As the amount of apoB in each particle of TG-rich lipoprotein is constant, the ratio of RP/apoB reflects the average amount of RP per TG-rich lipoprotein. If RP is not contained in apoB-100-containing TG-rich lipoproteins, then the ratio of RP/apoB-48 reflects the average amount of RP per particle of intestinally made TG-rich lipoproteins. **Figure 3** shows the ratio of RP to apoB-48 (both expressed in molar terms i.e., μmol/L) before and after heparin injection. The ratio measured in S_f 20–60 (8.1 ± 1.2) was significantly lower than the ratios calculated in $S_f > 400$ (69.3 ± 7.2) and S_f 60–400 (52.4 ± 7.7) fractions ($P < 0.05$). Figure 3 also shows that in each TG-rich lipoprotein fraction, the RP/apoB-48 ratio increased after heparin had been injected.

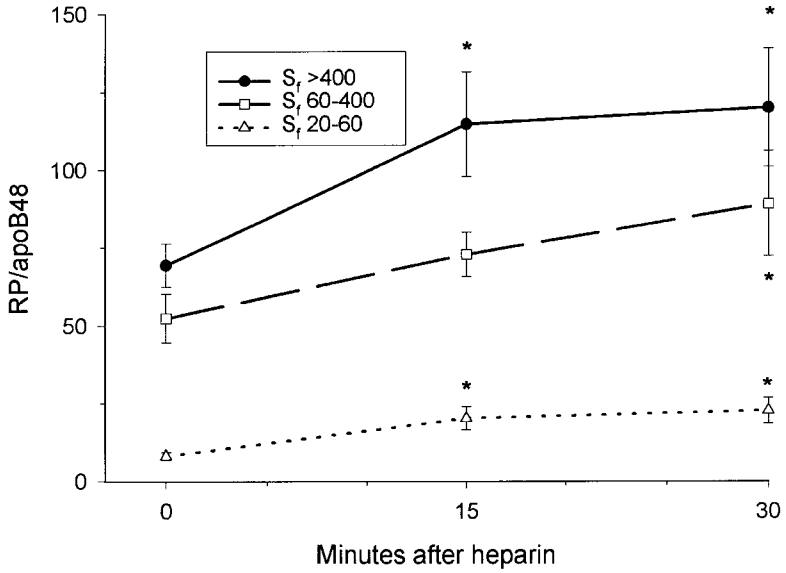

Fig. 3. Ratio of retinyl palmitate to apolipoprotein B-48 measured before the heparin injection and 15 and 30 min after the injection in $S_f > 400$, S_f 60–400, and S_f 20–60 fractions. *Significantly different from the ratio measured before the heparin injection ($P < 0.05$).

Retinyl palmitate in apoB-100-containing triglyceride-rich lipoproteins

Finally, the TG-rich lipoproteins ($S_f > 20$) obtained from 4 subjects who had participated in the postheparin study was used to determine whether RP was associated with apoB-100-containing TG-rich lipoproteins. The whole TG-rich lipoprotein fraction (containing 3.18 ± 0.24 mg of apoB-100, 19.52 ± 2.88 μmol of RP, and 3.72 ± 0.60 μmol of TG) was applied to an immunoaffinity column (see Methods). The sodium thiocyanate eluated contained 62.4 ± 4.0% of the applied apoB-100 (1.98 ± 0.16 mg), 3.1 ± 0.3% of the applied RP (0.57 ± 0.03 pmol), and 26.0 ± 4.5% of the applied TG (0.95 ± 0.24 μmol). Thus, 62.4% of the apoB-100 but only 3.1% of the RP that had been in the TG-rich lipoproteins applied to the column was recovered in the eluate. Therefore, at most, only 5% (i.e., 3.1/62.4) of the RP in the TG-rich lipoproteins was found in this apoB-100 fraction. The rest of the RP was, presumably, in the apoB-48-containing TG-rich lipoprotein fraction. Similarly, it appeared that 42% of TG was associated with apoB-100 particles.

DISCUSSION

Retinyl palmitate concentrations measured after the ingestion of a high fat meal supplemented with vitamin A are frequently used to study the metabolism of postprandial intestinal lipoproteins (2, 4, 7, 23–28). Measurements of apoB-48, which is associated with TG-rich lipoprotein secreted by the intestine, have also been utilized, although to a lesser extent, to follow these postprandial lipoproteins (15, 17, 29, 30). In theory, apoB-48 and RP should remain associated from the secretion by the intestine until the uptake of the lipoprotein by the liver and should therefore yield similar information about the metabolism of postprandial lipoproteins. Results provided by our studies challenge this theory.

In the postprandial study, we have shown that the peak RP concentration was delayed compared to peaks of apoB-48 and TG levels. This difference was seen in the three TG-rich lipoprotein subfractions studied and was most striking in the S_f 20–60 fraction. These findings are concordant with those of Karpe and collaborators (17) who have reported a delay in RP peak, especially in the S_f 20–60 fraction. Other studies, in which RP and apoB-48 were measured in the total fraction of TG-rich lipoproteins have also observed such a delay (15, 16).

Some have suggested that RP may exchange very slowly between apoB-48 and apoB-100-containing lipoproteins. They raised this possibility because they found that 3 h after a fat load, 9% of the total plasma RP was found in LDL (15). After 9 h this percentage went up to 19%. Cohn and colleagues (31) have also reported that up to 25% of the total RP response measured during 12 h after a high fat meal was accounted for by lipoproteins containing apoB-100. The presence of RP in lipoproteins containing apoB-100 could also be explained by the ability of the human intestine to produce apoB-100. This hypothesis received some support from studies which have demonstrated that the human intestine has the capacity to synthesize and secrete apoB-100 (32, 33). However, this is unlikely to play a major role as the proportion of newly synthesized apoB-100 is very low (3–5%) compared to apoB-48 (32). Furthermore, we have shown, in this study, that only 5% of RP associated with TG-rich lipoproteins was found in apoB-100-containing lipoproteins isolated from plasma drawn 5 h after the fat load. Therefore, in our studies, any exchange of RP from apoB-48 to apoB-100-containing lipoproteins or any secretion of intestinally derived apoB-100 lipoproteins was probably too little to influence our conclusions. Hence, we concluded that RP was in apoB-48-containing particles. We have also shown, using the immunoaffinity column, that 5 h after the fat load about 42% of TG present in $S_f > 20$ was associated with apoB-100 particles. This is concordant with results reported by Cohn et al. (31).

As mentioned, theoretically, apoB-48 and RP should follow similar postprandial patterns of appearance and disappearance. The concentration versus time curves (Fig. 1) and the times at which peak concentrations occurred (Table 2) suggested that this was not the case. In fact, the apoB-48 concentrations declined at a time when the RP levels did not. These observations raised two possibilities. Fat absorption could have continued without the production of apoB-48-containing TG-rich lipoproteins. This is considered unlikely. The other possibility is that there is some difference in the clearance of apoB-48 compared to that of RP.

We, therefore, undertook a second series of studies in which the clearance of TG-rich lipoproteins was examined. Previously, Berr, Eckel, and Kern (34) studied the in vivo decay of two components of intestinal lipoproteins: TG and RP. They found that lipolysis stimulated by heparin did not affect the clearance of RP, whereas TG decay was significantly enhanced under these conditions (34). To our knowledge, no study has compared apoB-48 and RP metabolism after in vivo stimulation of lipolysis with heparin. In our study, we loaded the postprandial TG-rich lipoproteins with RP in vivo by giving an oral vitamin A-containing high-fat challenge. Five hours thereafter, LPL-mediated clearance was stimulated by giving an intravenous bolus of heparin. As anticipated, after heparin administration, TG concentrations decreased significantly in the three TG-rich lipoprotein fractions. However, the responses of RP and apoB-48 to heparin were very different. After the heparin injection, apoB-48 levels in the $S_f > 400$, S_f 60–400, and S_f 20–60 fractions changed in parallel with TG levels. On the other hand, RP levels did not decline after the injection of heparin. This was particularly surprising as most have assumed that RP marks intestinal lipoproteins, i.e., the apoB-48-containing lipoproteins. However, the postheparin levels of apoB-48 and those of RP clearly followed different patterns.

The ratio of RP to apoB-48 measured before the heparin injection was lower in the S_f 20–60 fraction than in the more buoyant fractions. This finding is similar to that of Karpe and colleagues (17). They interpreted it to indicate that the apoB-48-containing TG-rich lipoproteins in

the S_f 20–60 fraction contained less RP per particle than those that are more buoyant. However, as the RP/apoB-48 ratio in a given fraction is an average value for the whole population, it is also possible that the population is heterogeneous. Some particles could be RP-rich and others could be RP-poor. This could occur if there were a mixture of apoB-48-containing (i.e., intestinal) TG-rich lipoproteins in the postprandial plasma, some from before and some from after the fat load. Such heterogeneity would presumably apply to the entire TG-rich lipoprotein population. It would mean that the intestinal TG-rich lipoprotein population contains RP-rich particles that represent those that are "new" and that contain recently absorbed lipids, as well as RP-poor particles that were present before the fat load and that are "older." The higher RP/apoB-48 ratio in the more buoyant TG-rich lipoproteins would then mean that the proportion of "new" particles is higher in them than in the denser population.

The differences between the patterns of apoB-48 and RP levels after heparin might theoretically arise from the ongoing absorption of RP after the heparin was administered. However, this is unlikely as the responses and differences in apoB-48 and RP were seen within 15 min of giving heparin and as it was 5 h since the fat load. Hence, the differences between the two probably reflect differences in their removal. There is still controversy about the effect of LPL on RP. Blaner and colleagues (35) have shown that, in vitro, LPL from fresh bovine milk was able to hydrolyze retinyl esters. If such a phenomenon would occur in vivo, it would tend to decrease the RP levels more than apoB-48. We observed just the opposite in our studies.

As heparin stimulated the removal of apoB-48 and TG more than that of RP, our data could suggest that heparin-stimulated lipolysis promoted the removal of TG-rich lipoproteins that did not contain RP or contained lesser amount of RP in preference to those that had more RP per apoB-48 particle. Indeed, the preferential removal of apoB-48 particles that contain no or a lesser amount of RP would explain the minimal change in RP levels compared to apoB-48 concentrations that we observed. This could especially be true in the S_f 20–60 fraction which may contain the most apoB-48 particles without RP (older particles) or with fewer RP per particle as suggested by Karpe et al. (17).

After heparin, the absolute levels of RP in the S_f 20–60 fraction increased. It would be tempting to attribute this increase to a contribution of larger particles which entered the S_f 20–60 fraction after lipolysis. However, the design of our study did not permit us to measure the transfer of TG-rich lipoproteins from larger to smaller fractions.

The preferential removal of apoB-48-containing lipoproteins that were in the circulation prior to the ingestion of the meal (i.e., "older" particles containing no RP) could be explained by differences in the composition of apoB-48-containing lipoproteins found in the fasting state compared to those in the postprandial state. Perhaps some compositional changes, for example in apoE or apoC, could make postprandial lipoproteins poorer substrate for LPL. Karpe et al. (30) have reported that in hypertriglyceridemic subjects, the increase in apoB-48 and apoB-100 observed in the postprandial state was not paralleled by increased concentrations of apoC-II. As it has been suggested that the amount of apoC-II relative to the total surface of the lipoprotein was a critical determinant of LPL efficiency (36), these changes in TG-rich lipoproteins observed after ingestion of the meal (30) may reduce the ability of postprandial lipoproteins to be hydrolyzed by LPL. Additional studies are warranted to clarify this issue.

In summary, results reported in this paper have shown that the pattern of changes observed in RP and apoB-48 concentrations after a high fat load are different. These differences could arise from differences in secretion patterns of apoB-48 and RP and/or from differences in their clearance from the circulation. Our studies support the latter. They suggest that the postprandial apoB-48-containing TG-rich lipoproteins are compositionally heterogeneous and also may be metabolically heterogeneous. The data raise the intriguing possibility that some properties of the TG-rich lipoproteins that have been circulating for longer times may make them more susceptible to LPL-mediated clearance.

We conclude that RP and apoB-48 do not mark the same properties of postprandial intestinal lipoproteins. It is probable that the apoB-48 should be interpreted as reflecting the intestinal lipoprotein particles, whereas RP would reflect lipid absorbed from the intestinal tract. Our study demonstrates that the physiologic meaning of one of these markers should not be extended to the other.

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